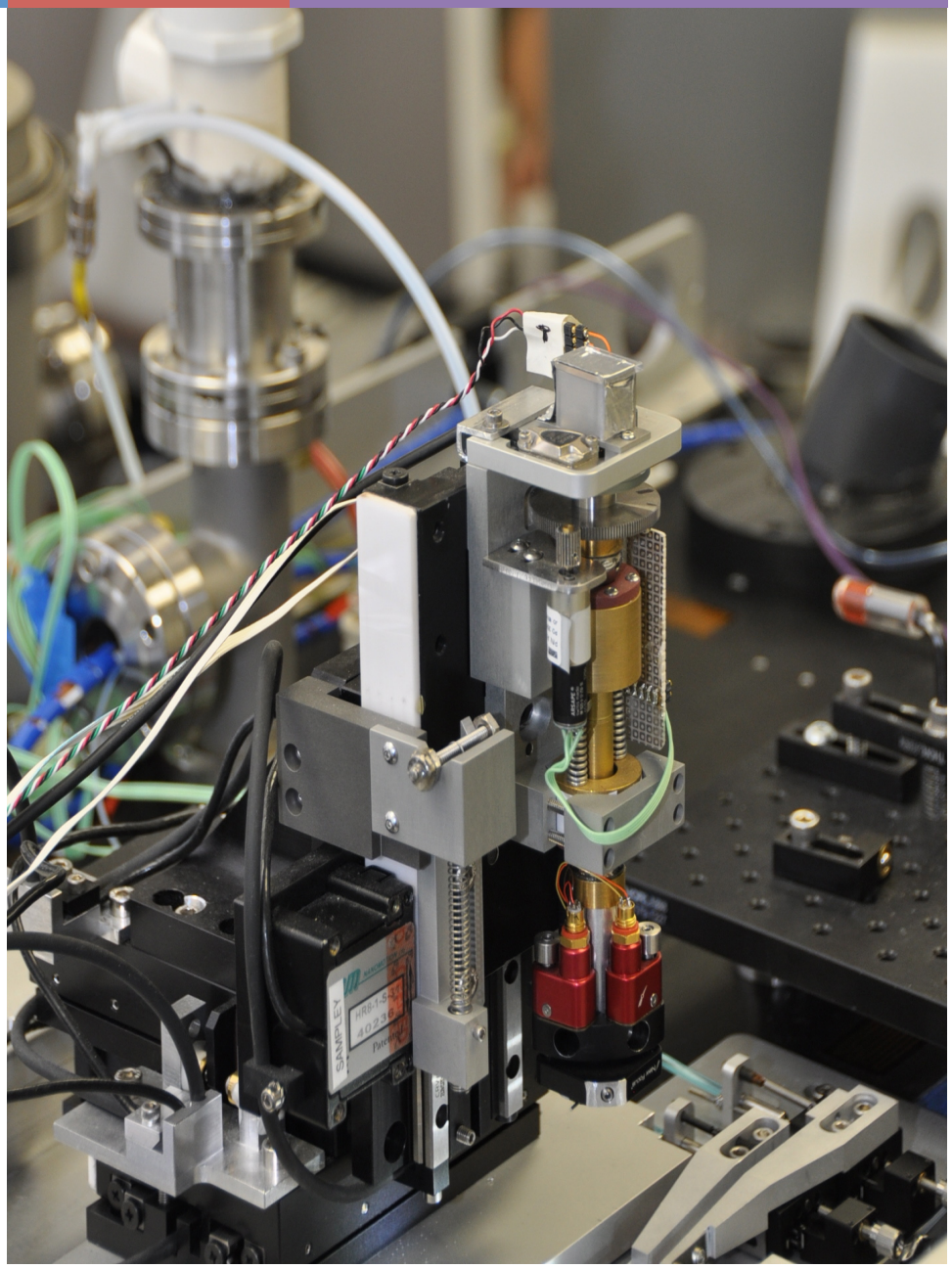
National Center for X-ray Tomography

Soft X-ray Microscope

User Information and Operating Manual

Bertrand Cinquin, Dec 2012



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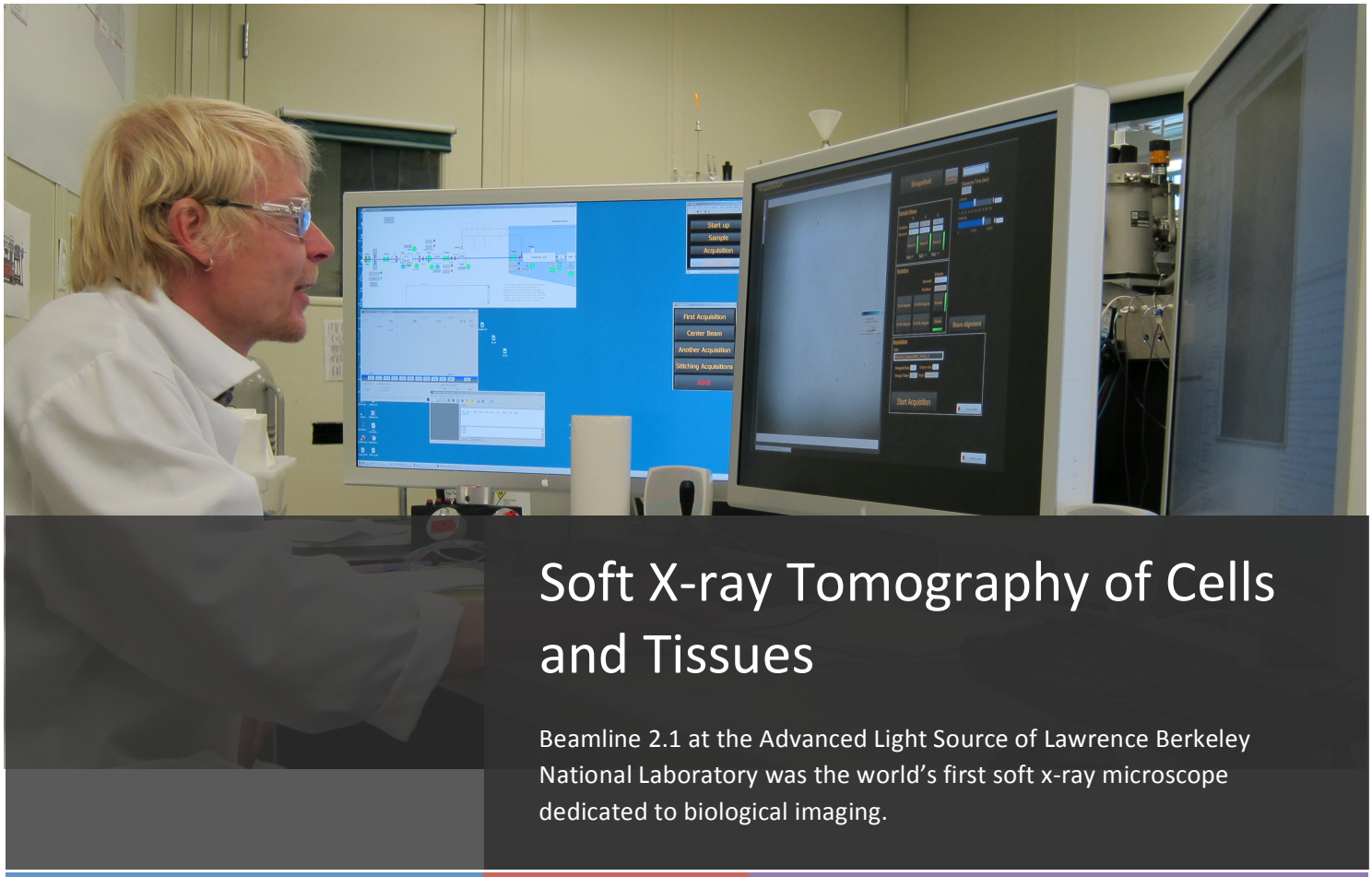


Soft X-ray Tomography of Cells and Tissues

Beamline 2.1 at the Advanced Light Source of Lawrence Berkeley National Laboratory was the world's first soft x-ray microscope dedicated to biological imaging.

This manual covers the basic operation of Beamline 2.1, a soft x-ray microscope for biological research at the Advanced Light Source.

Introduction

The National Center for X-ray tomography (NCXT) soft x-ray microscope is located in sector 2 of the Advanced Light Source. Currently, this is the only beamline operating in this sector of the synchrotron, and therefore should be relatively easy to find. The specimen preparation laboratories are located nearby on the 2nd floor of building 80 in rooms 80-210, 80-203 and 80-208.

Safety

Safety is a paramount consideration when using both the ALS and the facilities of the NCXT. Consequently, all users must complete appropriate safety training courses before they can gain access to the facility. Contact NCXT staff to determine which training courses are required for your particular experiments, or to determine your current training status if you are revisiting the facility. Note: Although you may have completed training courses for earlier visits some courses need to be retaken every year, or may be required for specific experiments or specimens.

Once you arrive at the ALS an **experimental user safety sheet** must be signed prior to the start of every data collection run on the x-ray microscope. The experiment safety sheet documents who is using the facility at any given time, and ensures that everyone who takes part in an experiment is correctly certified.

Safety is our highest priority

NCXT and ALS staffs are here to help you accomplish your experimental goals, and work in complete safety. If you have any doubts or questions about your work, or the work of those around you please contact a staff member immediately.

Emergency services – paramedic, fire and security – are located adjacent to the ALS. They can be contacted any time night or day. Please don't hesitate to call for assistance in case of fire, illness or any other emergency. **If in doubt, call anyway.**

7-911 from lab phone or 911 from a cell phone

For all other issues please call the ALS control room – extension 4969

Important locations: Knowing your way around the ALS makes your experimental time as effective as possible. It is also highly recommended that you familiarize yourself with exits, and the evacuation strategy in the event of an earthquake.

In case of an earthquake duck, cover and hold

Response if you feel an earthquake

- **Duck** – Drop to the floor
- **Cover** – Position yourself under a sturdy overhead protection, such as a desk, table, work-bench, or room corner
- **Hold** – Hold onto the protection you've chosen and prepare to move with it until the shaking stops
- Do not panic or run.

Evacuation

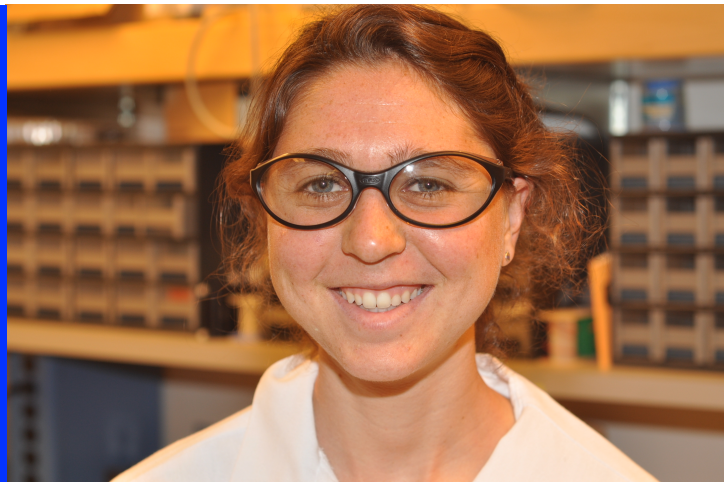
- Evacuate immediately. Use the evacuation routes and assemble in the designated areas clear of buildings and overhead structures.
- Take keys and other small personal belongings, as re-entry to the building may not be possible for days
- Follow Building emergency Team instructions
- Do not re-enter building without permission

Specimen Preparation

Liquid nitrogen can be obtained for the cold traps and cryostage from the 50 liter vessel located at the door to the x-ray microscope or, in the event that this supply runs out, from the large Dewar directly across the walkway from the beamline. BE SURE TO ALWAYS USE PERSONEL PROTECTION EQUIPMENT WHEN HANDLING CRYOGENS INCLUDING FACE MASK AND GLOVES.

At a minimum you should wear a lab coat and safety glasses when you are in any experimental area (x-ray microscope, bio-labs, etc).

Open toe shoes, shorts or a bad attitude towards safety are not permitted in the NCXT labs, or in the areas of the ALS with red flooring.



While we are on the subject of safety, please no eating or drinking in the experimental areas. In addition to putting yourself at risk of ingesting toxic chemicals, spilled food or liquids can cause expensive damage to sensitive instruments and equipment, or cause contamination. Please, resist the temptation!

Specimen preparation and Mounting Cells for Imaging

Biological cells vary enormously in terms of their needs, what is good for one specimen can be terminally damaging for another. Whilst we have some general strategies for handling and mounting cells each project is fine tuned to maintain optimal cell health. At the NCXT we have sufficient experience, expertise and instruments to deal with more or less any type of cell. Rather than describe this in great detail you are simply advised to talk to NCXT staff. They are happy to help ensure your experiment is a success.

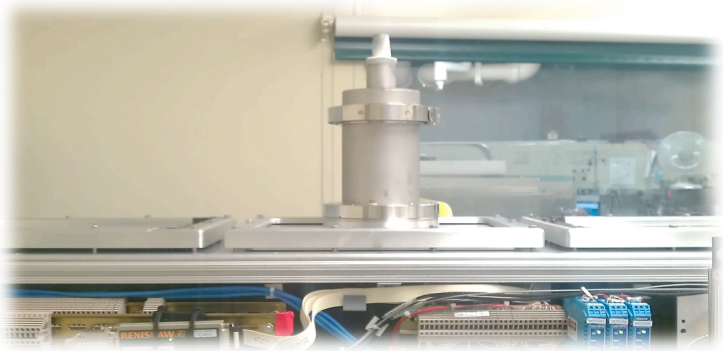
Operating the soft x-ray microscope

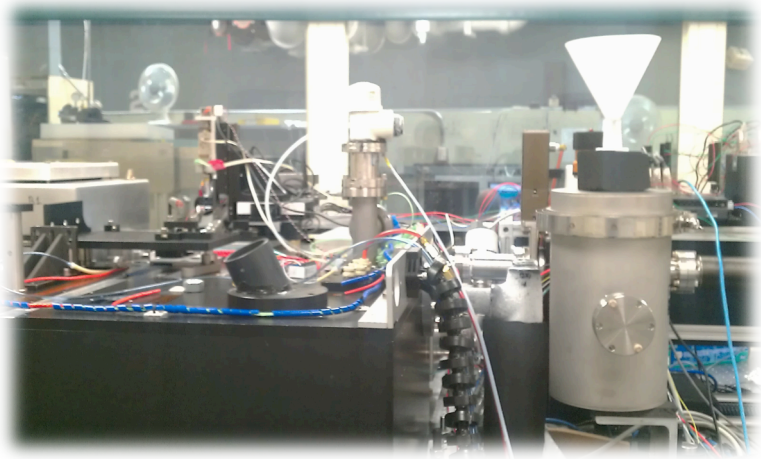
Operating the soft x-ray microscope

Beamline staff will provide you with a password for the x-ray microscope control computer and the NCXT General User Database (GUD). Please keep this password safe, and do not share this password, even with other people in your research group. If you forget your password please contact a staff member to obtain a new one.

Turning on the microscope

Before launching the graphical user interface first:
1) Fill the liquid nitrogen cold traps on the condenser box and the zone plate box.





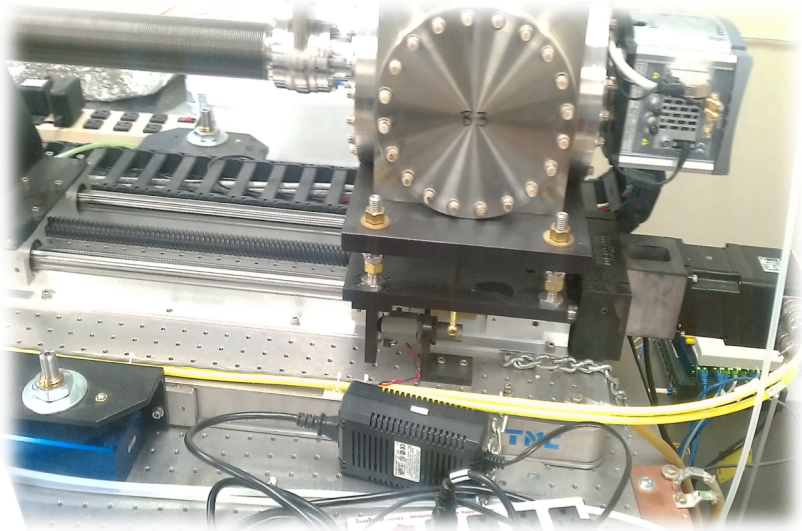
2) Turn on all the controllers for the microscope, starting with the shutter controller and condenser scanner.

Operating the soft x-ray microscope

Next switch on the power supply for the condenser box Galil controllers (Black box under the optical table) and the sample, MZP and He gas flow Galil controllers (Second black box under the optical table). Finally switch on two power switches for the alignment cameras, the temperature controller the x-ray CCD thermoelectric cooler and the newfocus motors.

Once all necessary hardware has been switched on activate the LabView GUI (shortcut on the beamline desktop computer called ‘GUI’) and start the branchline control system called "Branchline EPS" (also on the desktop).

Start the General User Data Base (GUDB)



Operating the soft x-ray microscope

Before opening the valves on the branchline, check the pressure gauges on the beamline. If the pressure is not sufficiently low, indicators will be red and you will not be able to open the shutters (In which case, help from Mark Le Gros, or an ALS control room operator is required).

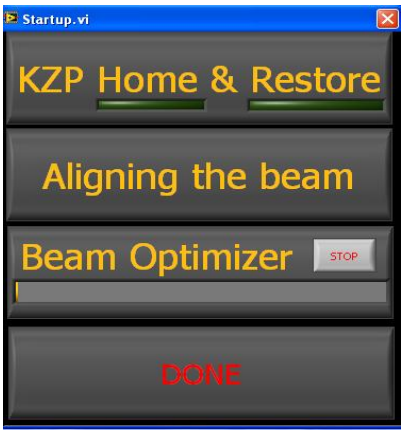
Pressure at IG207 should be around 10^{-5}, pressure at IG206 around 10^{-7},IG205 is around 10^{-8}, IG204 and IG203 are close to 10^{-10}. These pressures will change once the x-ray beam is open, with IG203 and 204 rising to low 10^{-9}.

You need to open the valves VVR204 (far right button) and VVR203 in order to open the VVR202 (far left button) to let the beam to come through into the microscope. The black line turns blue when the x-ray beam is present. If after trying to open VVR202, the button is blinking, you need to call "RING" and contact the ALS duty operator (extension 7464). In this situation another shutter needs to be opened by the ALS operators, this is not usually the case but sometimes after a long accelerator shutdown the beamline maybe offline.

Initial Microscope Alignment

These elements use a variety of different motors and encoders, and each element must first be homed to the correct position on starting the microscope.

First step in the procedure is pressing the "start up" button on the main GUI to open the Startup.vi control panel and then pressing the button "KZP Home & Restore".



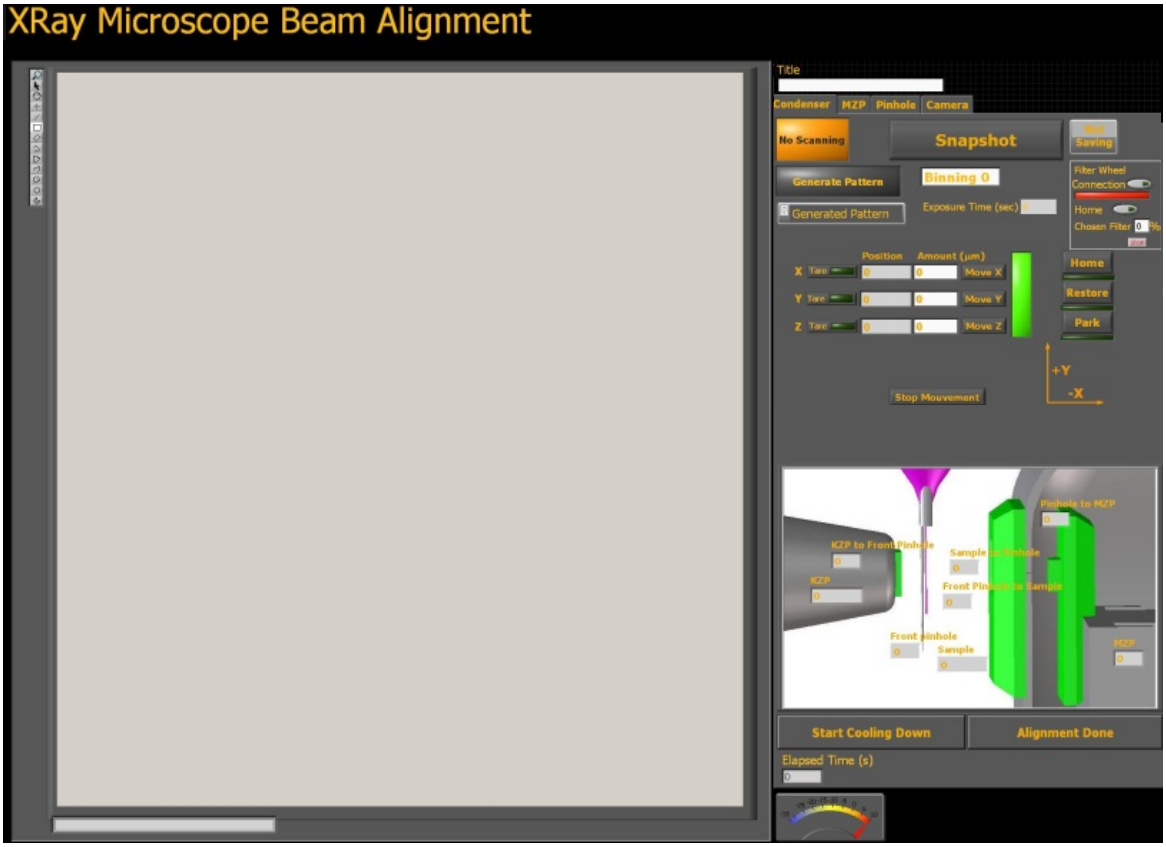
On initial startup several optical elements need to be moved to find the beam and optimize the throughtput. The major components are: The KZP (the condenser zoneplate lens) the front pinhole and the MZP (the objective zoneplate lens). The KZP has no absolute encoder and a homing step is necessary to establish the correct zeros of the coordinate system on each of the three axes. The second step is restoring the condenser to the last known optimal imaging position (this is achieved by activating "Startup → Home & Restore"). The restore action automatically picks up the last good KZP coordinates from the microscope database and moves the KZP accordingly.
Homing and Restoration takes several minutes.

The "KZP Home & Restore" indicators turn green when it is complete.

Initial Microscope Alignment

The position of the Pinhole and the MZP (x and y axis) is encoded by an absolute encoder (LVDT) and does not need to be initialized. The Camera can move on the optical axis but usually does not require movement during normal operation. (See troubleshooting section)

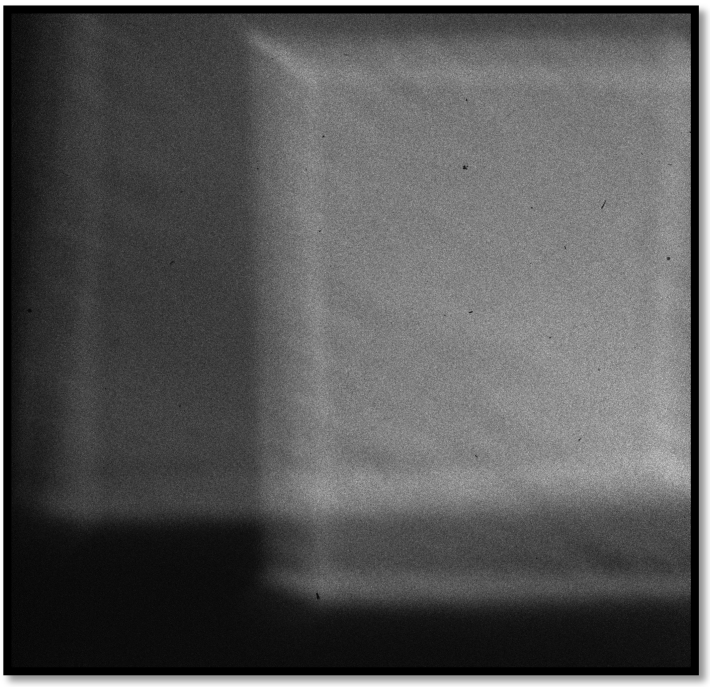
The second button on the Startup GUI, "Aligning The Beam" provides an interface that allows you to align the microscope to find, center and optimize the throughput of the beam on the camera chip.



The beam alignment module interface window opens on the right screen. This module gives you access to the optical elements described above. Images taken during alignment can be saved after entering a name, clicking on the button "Not saving" (This button toggles between "Saving" and "Not Saving" conditions). Exposure time can be selected by choosing between "No-Scanning" of the beam (50 ms) and "Scanning". Before taking any scanned image, you have to generate a pattern using the “generate pattern button”. Position of the KZP can be read out "Position" and the required displacements can be entered in the "Amount" window. Pressing the button "Move X" moves the KZP on the X axis by the X amount entered. Similarly for the Y and Z axis. Tare buttons are available to simplify tasks involving multiple displacements. You can directly untare to find the

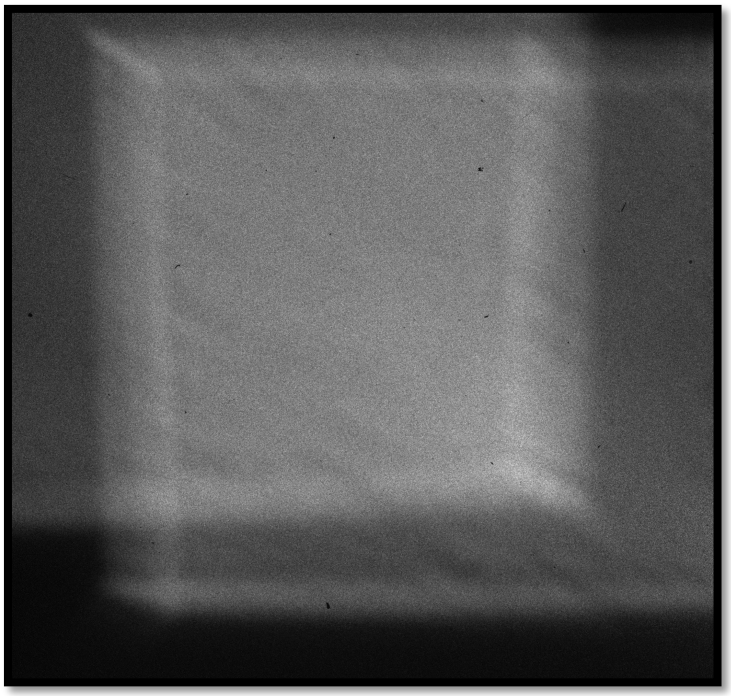
Aligning the microscope

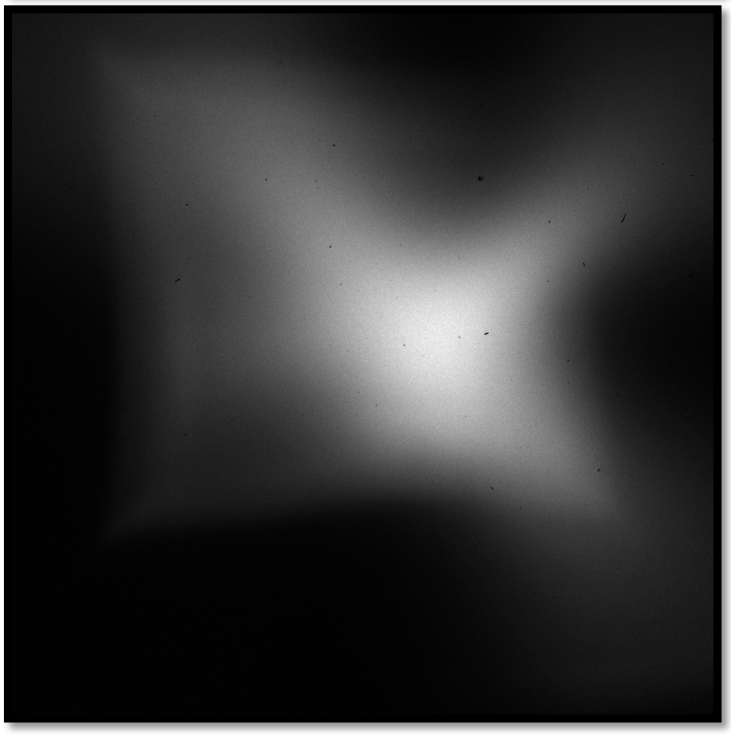
After choosing the "No Scanning" mode, take a snapshot by pressing the "Snapshot" button.
The Image should be close to the figure low: two squares out of focus. In case of a black screen, please check that the shutters (EPS system are opened) and beam is available. If it is still the case, please see the troubleshooting section.
You want to have the entire brightest square in the field of view of the camera. To do this, move the MZP. The maximum displacement for the MZP must not be more than 5 microns. Otherwise you risk losing all light coming through. X axis motion of the image is intuitive (moving the square image to the left requires a negative displacement, moving the square image to the right requires a positive movement). The Y axis is reversed (moving the square image to the top requires a negative displacement. Moving the square to the bottom requires a positive displacement)
A guide to the movement direction of the image when the MZP is moved is represented by the diagram on the "Aligning the Beam" GUI. (Summary of this diagram: +X = →, -X = ←, +Y = ↓, -Y = ↑).



Aligning the microscope

An image close to above image can be obtained. You need to notice what is the brightest side of the image and move the condenser spot to the center of the square.
Amount for the moves must not be over than 10 microns, 10 to 20 um total movement of the condenser is usually all that is required. For the condenser the X axis motion is reversed and Y axis is intuitive. To summarize the moves of the KZP:(+X = ← , -X =→ , +Y = ↑ , -Y = ↓)
After several displacements (rarely more than 100 microns on X and Y axis) You should obtain something like this :





The shape of the condenser spot looks like a square. Once the beam is centered, the cooling down procedure can be launched by pressing the “Start Cooling down” button. This will automatically increase the He flow to 0.6 (the far right pressure indicator on the pressure indicator board). You need to fill the white polystyrene keg with liquid nitrogen in order to flush the system. After 10-15 min, you can increase the pressure of Nitrogen gas to 30 and fill the cryobox with liquid Nitrogen. **You need to turn on the heaters**.

Cryostage gas flow controls

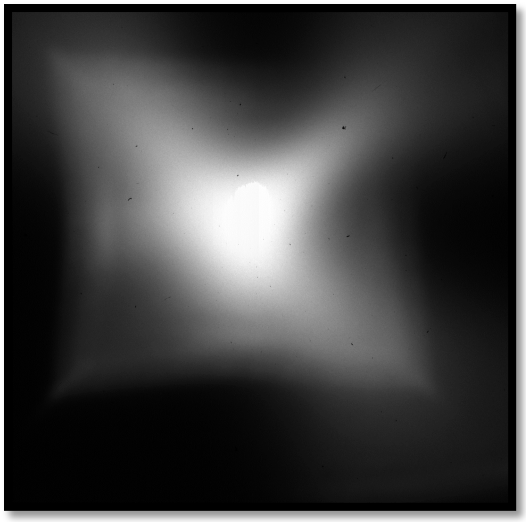
The temperature will drop slowly. You need to increase the flow of nitrogen in order to reach the cryo temperature (-189 Celcius on channel 1, -186 Celcius on channel 2 and -153 Celcius on channel 3).
Don't raise the flow of nitrogen gas too fast. This procedure can be followed:

Pressure of Nitrogen	Temperature
30	-60
45	-120
60	-180

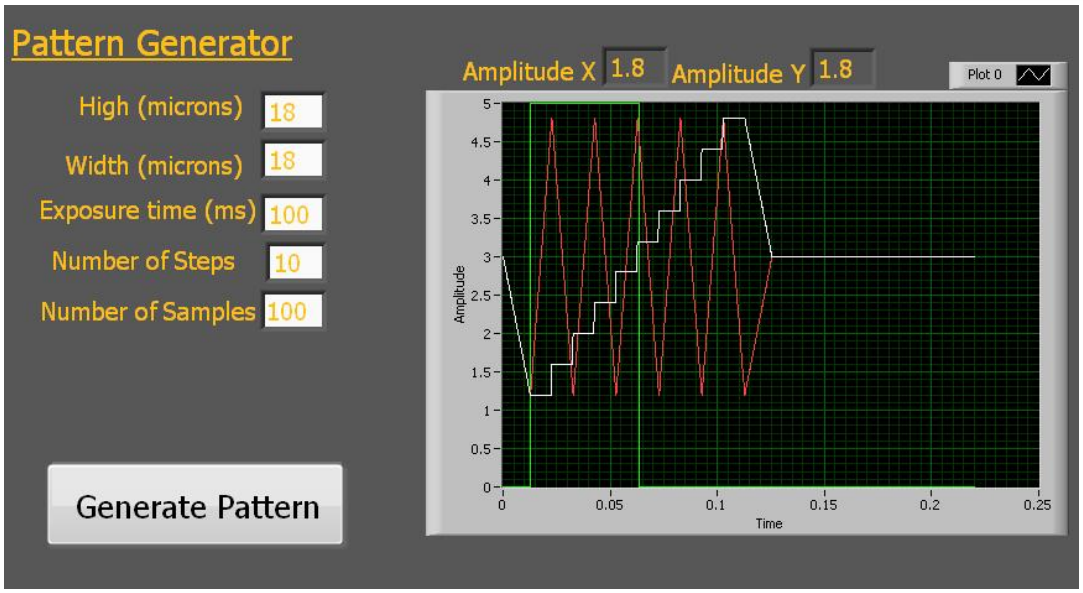
Temperature can be read out to the computer desktop using the "ScanLink 2.0" software. During the cooling down process, you should take some images. Because Helium is flowing around the sample environment, less absorption occurs (due to Nitrogen and Oxygen mostly). You should obtain an image similar to this.
Saturation is possible but causes no harm to the camera. The MZP and KZP may require minor adjustment as the system cools. To optimize the beam, an automatic alignment procedure is available. It will optimize the pinhole position in order to have the maximum light as possible. **It should only be operated when the microscope is cooled down.**

Condenser scanning parameters

Without condenser scanning (no scan 30 ms exposure) image should be similar to the figure on the right.



In order to have a homogenous throughput, the condenser moved following a given pattern, pattern programmed by the Pattern Generator. The first scanned image needs this pattern. The program will encounter an error asking for the re-initialization of the camera



The pattern generator allows a simple lines scanned pattern. It is defined by the amplitude in X and in Y, the exposure time (time to make the scan), the number of steps and samples. These two last parameters are rarely modified. The graph presents the voltage variations of the different channels that draw the pattern (X in white, Y in red). In green is the pulse that will trigger both camera and the moves of the condenser according to the defined pattern. Here the result with a 100 ms exposure time and pattern of 18 by 18 microns with He flowing

Specimen loading

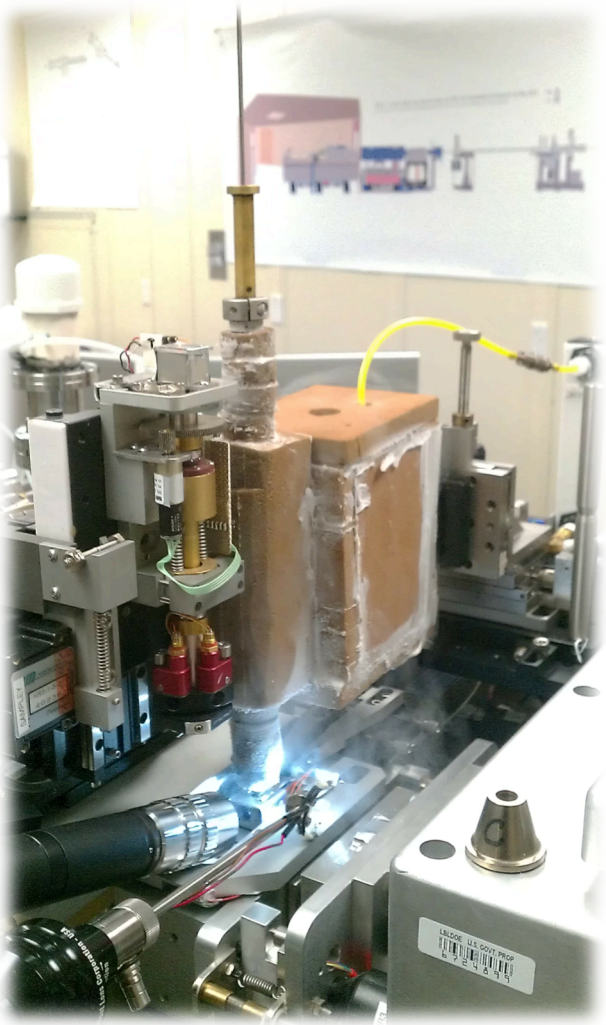
The sample stage has seven motors (regular motion axis X, Y, Z, two inclinations planes XY and XZ and the rotation around the Y axis).
The first step is to home the rotation motor. To do so, click on "Homing rotation". When the indicator becomes green the homing is complete. It is important for two reasons

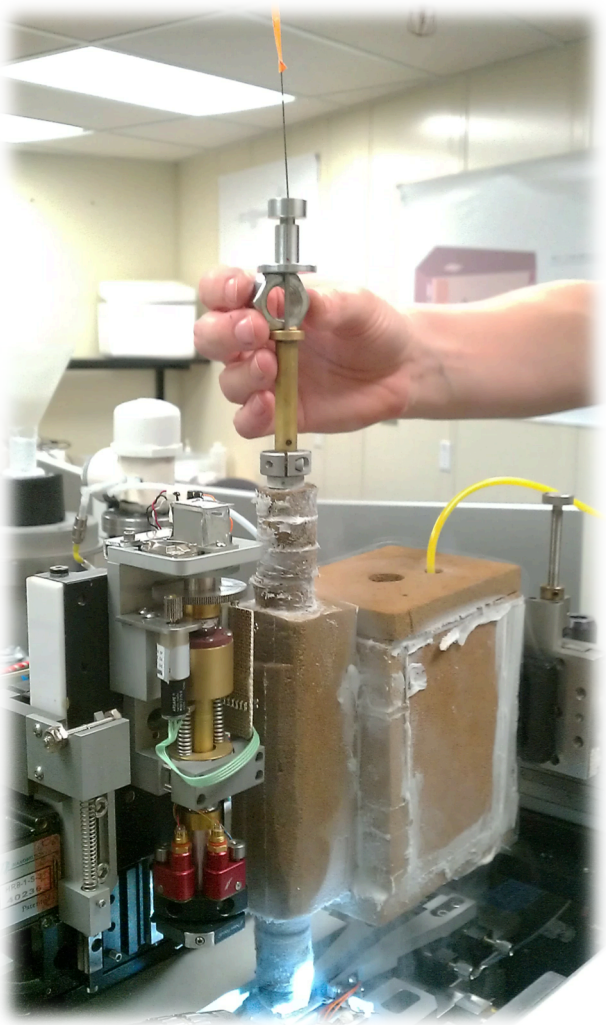
1. To start the acquisition with the right angle
2. Be at the right position for the cryotransfer of the sample.

When the indicator is green, you can press the "Loading New Sample" button.

Sample loading and alignment

1. The Helium flow rises to 2.5 (one the indicator. Voltage is about 5 V), a little column of cold helium can be observed coming out of the sample chamber.
2. The stage moved away by -50000 microns in X to allow room for the cryo transfer machine.

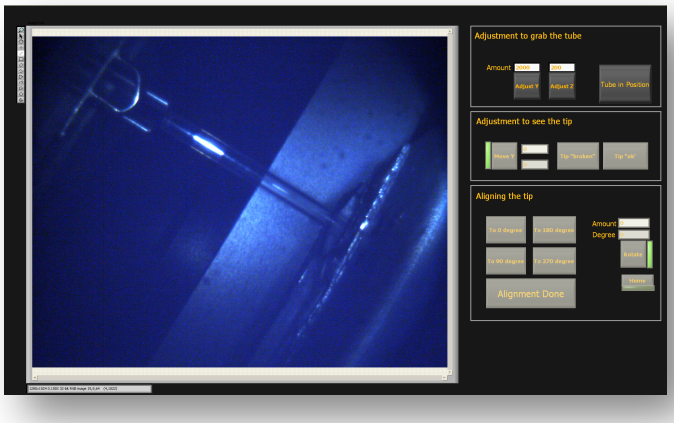


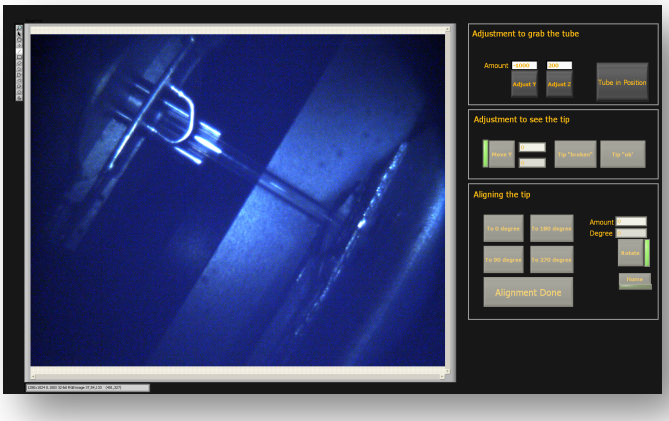


Specimen loading

1. To replace the transfer device, be sure it makes contact with the hard stops (metal pieces) and screw the side clamp to be sure of the stability of the transfer machine.
2. The clamps that allow the transfer between the machine and the sample stage are opened.
3. The program dialogue asks you to transfer the sample using the transfer machine. When the tube is down, click "Done". Use a bolt to prevent the tube to going too far down. It could contact the back pinhole.
4. The clamps close to grab the tube. The program dialogue asks you to remove the transfer machine. Don't click "Ok" before this is completely done.
5. The stage will move back to the specimen center and go down to approach the tube

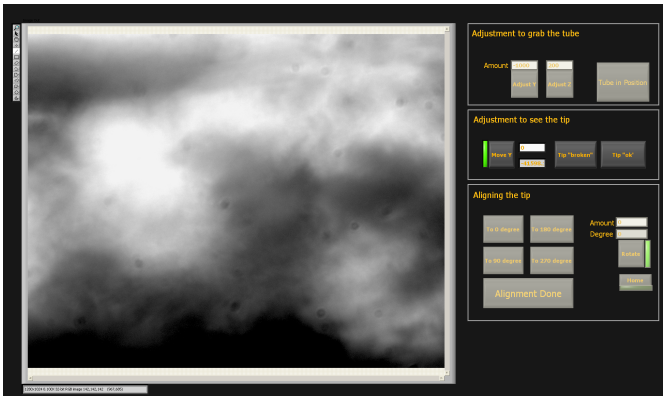
The camera of the boroscope is automatically connected to allow you to tune the stage in order to grab the tube. You can watch on the side if the sample holder (two poles) is slightly to the right of the tube. If it is not the case, you need to adjust z with 100 micron step. You need to adjust y by several steps of -1000 microns (between 5 to 7 steps). If you push the tube down, it is certainly because the stage is not at the right place in z. You need to go up a little bit (500 microns are enough) and adjust the stage position by 100 microns step on the z axis.





Specimen loading

6. When the stage is grabbing the tube by several thousand microns, you can click the button "Tube In Position" and clamp the tube with the little spring. Then press Ok. This causes the clamp to release the tube.
7. The GUI automatically switches to the side light microscope allowing a look at the tip and being able to answer these simple questions : Is the tip is ok? (i.e. not too thick, not too icy, not broken). If the tip is broken, click "*Tip Broken*" to take the tube out. You can start the procedure of loading again with a new specimen. To find the tip, you need to move the stage by around +17000 microns in Y.



The tube is not straight. The tip, particularly where the sample stands need to be perfectly aligned during the rotation. To do so, please follow the eucentric alignment procedure.

Eucentric alignment procedure (using the Rotary Optical Encoder)

1. Placing the tube in the middle of the screen and in focus (angle 0)
2. Look at the position at 180 (by clicking to 180 degree button). (Measure the distance roughly, you can draw a line to remember the position.
3. Move very gently by half the distance using the NewFocus Motor B (left and right)
4. Look at the position of the tube at 90
5. Look at the position of the tube at 270
6. Move by half the distance between the two last position using the NewFocus Motor C (up and down)
7. When the tube is in focus for every position and stays at the same position. You have well aligned the tube.
8. Before clicking alignment done, place the tube in the middle of the screen with the tip in focus. This will make it easy to find the tube under the X ray Beam.
9. By clicking alignment done, you close the loading module and the stage is going down by -21500 microns. The tube will be close to the position to be imaged with X rays.

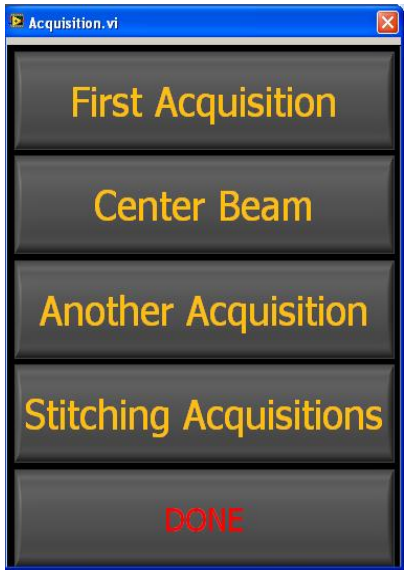
You are now ready to image the tube and acquire tomographic data. To do so, you have to close the "New Sample.vi" by clicking the button done.

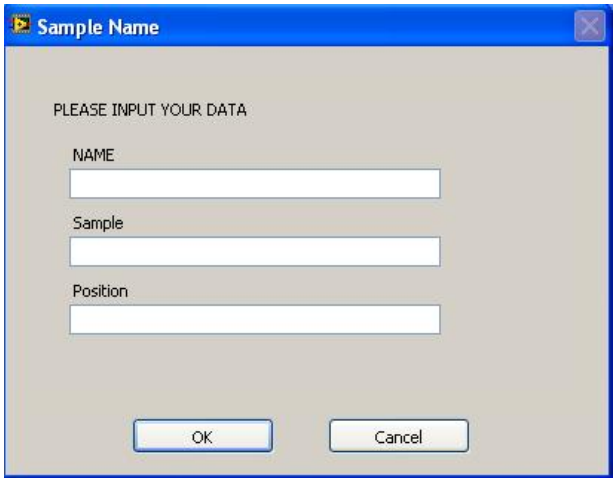
You need to click on the "Acquisition" to open the "Acquisition.vi". This will open the next module.

X-ray image alignment and data acquisition

Once the tube is aligned in the optical microscope you can launch your first acquisition by clicking on first acquisition. A window wills pop-up asking you to enter some information concerning the sample.
A folder will be automatically created where the data will be saved. This folder will be called Name_Sample_Position_01 and the name of the files: *Name_Sample_Position_01_xx.tif*

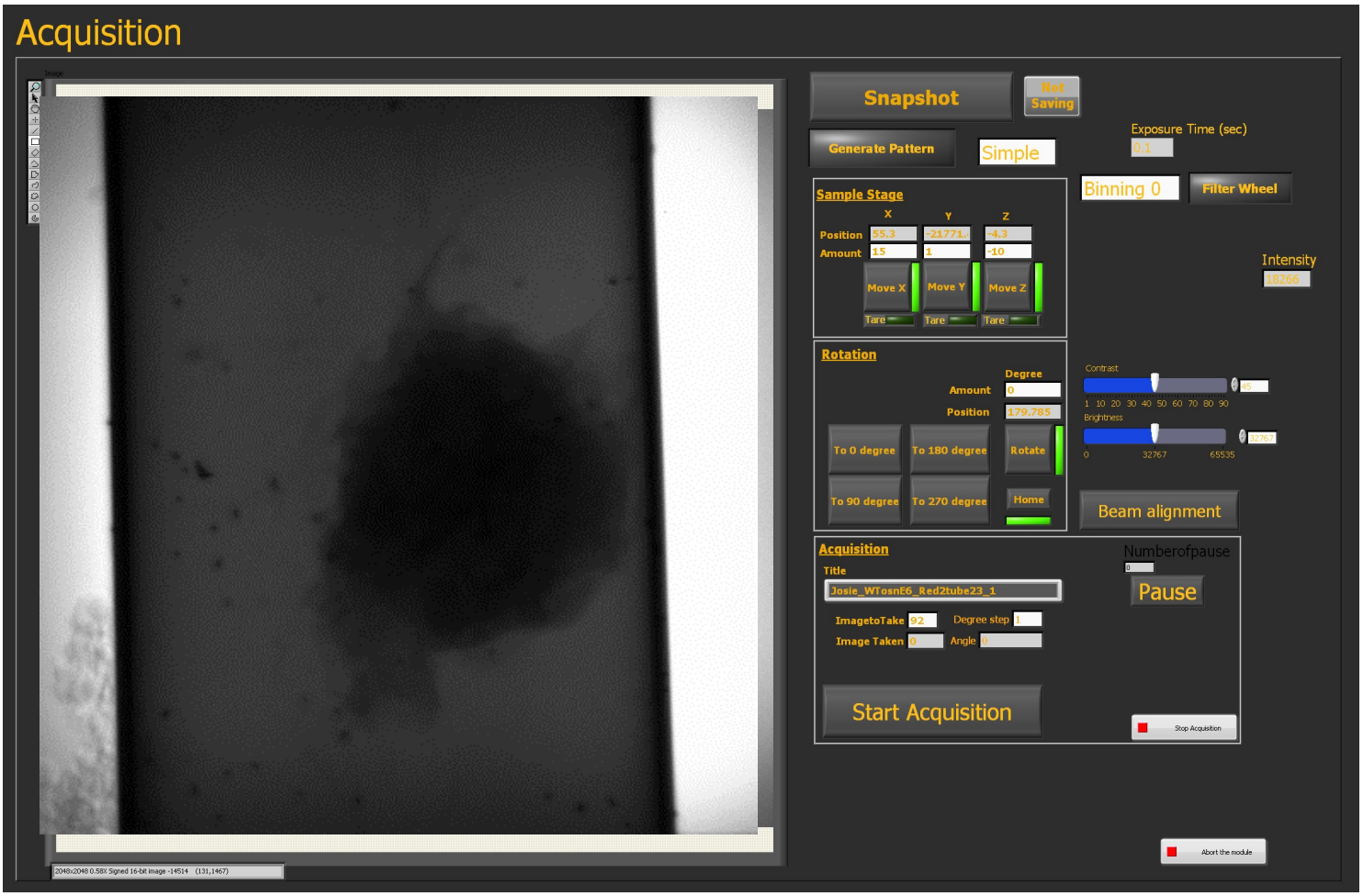
The acquisition GUI window appears on the right computer screen.





Warning : Before taking any snapshots, you have to generate a pattern as described previously. This is needed the first time you open the module only

X-ray image alignment and data acquisition



You can save snapshot images by toggling the button “not saving”. It will change for saving. The images will be saved at C:\essaiimage\Name_Sample_Position_01_xx.tif. The exposure time box enables a choice between different scanning patterns: simple (exposure time defined by the pattern generator) or double (double exposure time). In this module, you have access to all the motors of the sample stage, X Y, Z axis and the rotation motor. The bottom panel presents the parameters for the acquisition. Number of steps (number of images), the step value (degree). A Pause button available during the acquisition will give to the user the possibility to pause the acquisition and replace the tube in case of misalignment or drifting of the tube. The button “Beam alignment” opens another window to allow fine tuning of the image. You only have access to the X and Y axis of the KZP and the MZP in this alignment module.

An intensity indicator allows an effective reading of the intensity value of the image disregarding the brightness and contrast chosen by the two blue bars.
A Filter wheel button allows the choice between different neutral filters in order to tune the throughput. (This is only needed for expert in order to check the quality of the optics by comparison with a reference).

X-ray image alignment and data acquisition

The acquisition module performs several tasks in order to collect good projection data for tomographic reconstruction. As an example the projections image data needs to be normalized by a Flat field image (images of the field of view with a single scan with no specimen present). The sequence is as follows

1) The stage moves away by 40 microns.
2) 10 flat field images are taken
3) The stage moves back by 40 microns
4) A popup windows ask you to select your scanning pattern between a single scan and an double scan (lines only).
5) The acquisition of your specimen projection data then begins.
6) A pop-up window tells you when the acquisition is done. By clicking OK, it will close the right panel.

You will find your files into C:\EssaiImage\ Name_Sample_Position_01. Inside you will find the n images of the acquisition from Name_Sample_Position_01_01.tif to Name_Sample_Position_01_n.tif, two folders called FF1 and FF2 containing the 10 first flat field images and the 10 second flat field images and a txt file (extension rawtlt). This file records the different angle for each projection images taken. It will be useful for the reconstruction using the IMOD program. One can now use "Another Acquisition" button to take another data set on the same tube. The difference between first acquisition and another acquisition is only the fact that the name of your new acquisition will be Name_Sample_Position_02. The number is automatically incrementing by 1. If you need to enter a new name or sample name, you have to select first acquisition again.

When you want to load a new sample. You have to close the Acquisition module by pressing "*Done*", then open the sample module on the main GUI. Click on "*Unloading*" before loading a new sample. When you don’t want to image anything else, you have to close the Acquisition module by pressing "*Done*" then click on the "*Shutdown*" button.

Stitching acquisition routine

The stitching acquisition module is very similar to the previous one.
Several parameters are selectable, such as the number of field of view you would like to stitch, the displacement in Y between each field of view.
A beam alignment module will open between each acquisition to give time for the user to check the level of nitrogen in the different tank and of course realign the beam in case of drifting.

Shutting down the microscope

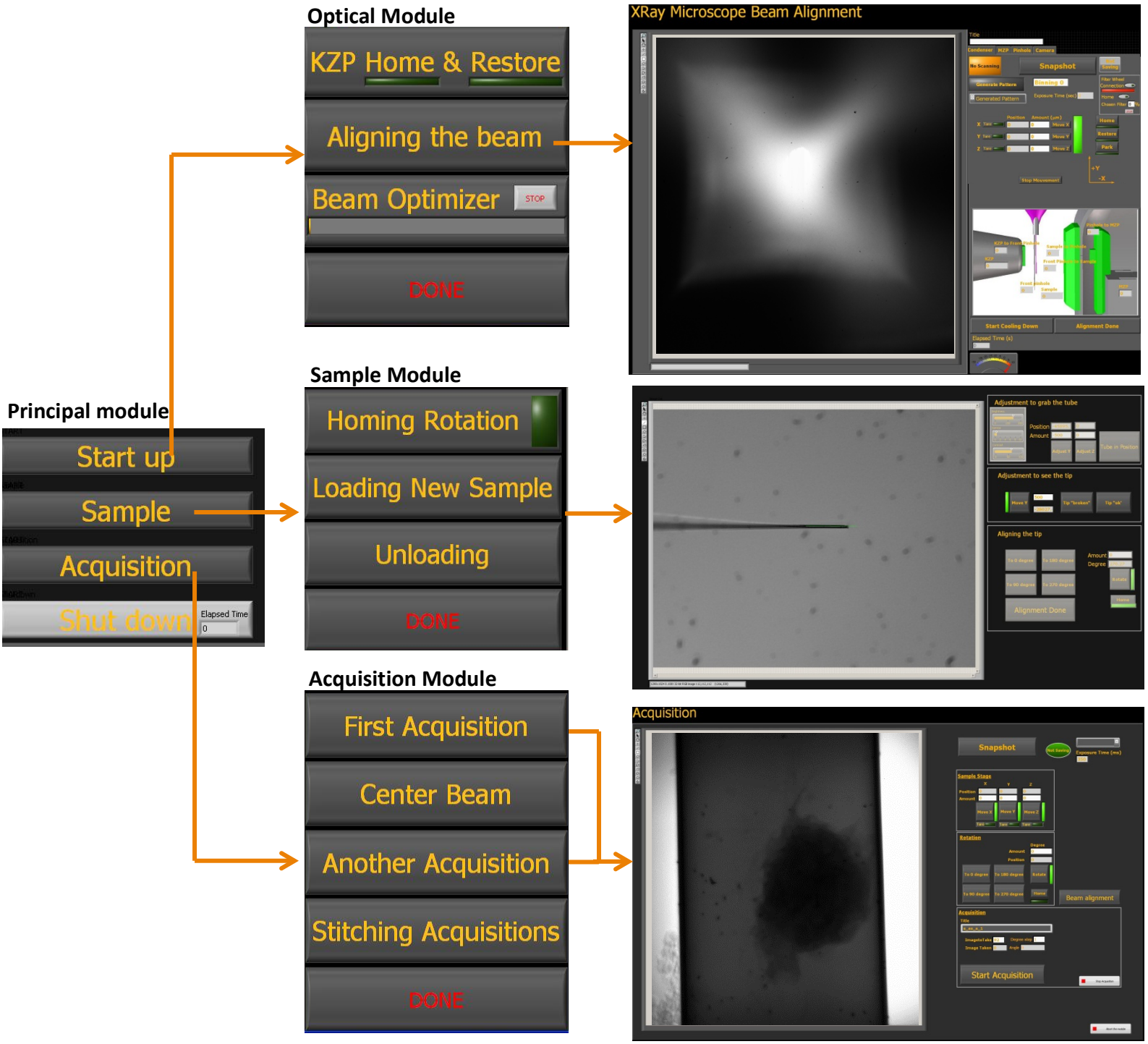
Automatic procedure

By clicking the *Shut down* button, you will launch the warming up and shutting down process.
It will automatically save the position of the pinhole, the MZP and the KZP, this module then parks the KZP and warms up the camera. The module will unload the sample if you forgot to do it after the last acquisition. The Vi will automatically stop when the camera reaches ambient temperature. This is the end of the automatic procedure; manual procedures are required for the remainder of the shutdown.

Manual procedure

A few things need to be done manually. Decrease the flow the Nitrogen to 0. Be sure that He is flowing in order to flush the microscope with dry helium. You can unplug the cooler controller of the camera when the Shutdown vi stops. You need to wait until the temperature of channel 1 is positive then you can close the He gas cylinder and the Nitrogen bottle. You can shut down the different controllers, power switches, the shutter box and the heater controller. Closing the valve of the zeolite cold trap.

Overview & Summary



Troubleshooting

During data collection you may encounter issues with the microscope. Most can be fixed easily. However, if you are not confident in what you are doing, or have any doubts…STOP and call for help! Whilst precautions have been put in place to prevent injury to you, or damage to the instrument it is impossible to envisage and account for all possible scenarios that can occur in a complex scientific environment. Help is free. On the other hand repairing damaged optics and instruments is expensive. Please don't take chances that endanger your wellbeing, the safety of others, or the integrity of the instrument.

Q. What if you don't see any specimen illumination light?

If you don't feel confident contact a staff member. If you are familiar with the microscope and the control system, and confident of your abilities then work through the following list of possibilities:

- ✓ Check the EPS windows. Are the shutters opened?
- ✓ Check beam is available and the synchrotron ring is operating normally
- ✓ Check if the shutter control box is switched on (this is the black box on the left on the optical table)
- ✓ Check if the KZP has been restored to the previous position. To do this check the database from where the data are collected and saved (when the microscope is turned off). The database can be found in C:\Database\DatabaseGalil.accdb. It will be opened in Microsoft Access 2010. The table "Galilparameters" can be opened by a double click. You will find the different position of the optical elements according to the date. The last line is the last known position when the microscope was operated. You can directly check if these numbers are consistent with the read positions at the different tabs of the alignment module. If you notice a difference, you can try to move the elements (KZP, Pinhole and MZP) to this previous position. **Warning: Don't ever move the MZP along the Z axis**. Call Bertrand Cinquin or Mark Le Gros to assist with this problem.
- ✓ Go the camera Tab, home it and move it by several steps of -100000 microns (-700000 maximum. It will move the camera toward the head of the line to have a larger view of the optics (you will see some photons of the 0 order passing around the condenser). Call Bertrand Cinquin or Mark Le Gros before proceeding with data collection.

Q. Some popup windows appeared with the error message ERR-2147353267

- ✓ Check the controllers: Maybe some of them are not responding. You need to turn them off and back on again before trying again.

Q. Some popup windows with the appeared ERR-2147467259

- ✓ The instrument is loosing connection with one of the controllers. You should restart all of them.

Q. The GUI is active but clicking any buttons does not active anything

- ✓ Labview Vis can't be simply aborted by being shut using the cross (right corner of a windows window). You have to close the different modules using the Done button.

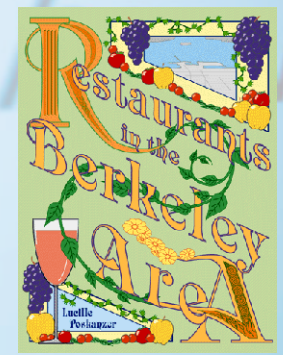
Useful websites

The Advanced Light Source

www.als.lbl.gov

University of California San Franscisco

www.ucsf.edu



Of course, you shouldn't come to the Bay Area without sampling the scenery and the food. Visit www-rnc.lbl.gov/Restaurants/ for a list of Berkeley restaurants compiled by Lucille Poskanzer.

Alternatively, if you have more time you could catch BART into downtown San Francisco. Trains leave regularly from the BART station in downtown Berkeley. Visit www.BART.gov for the schedule.

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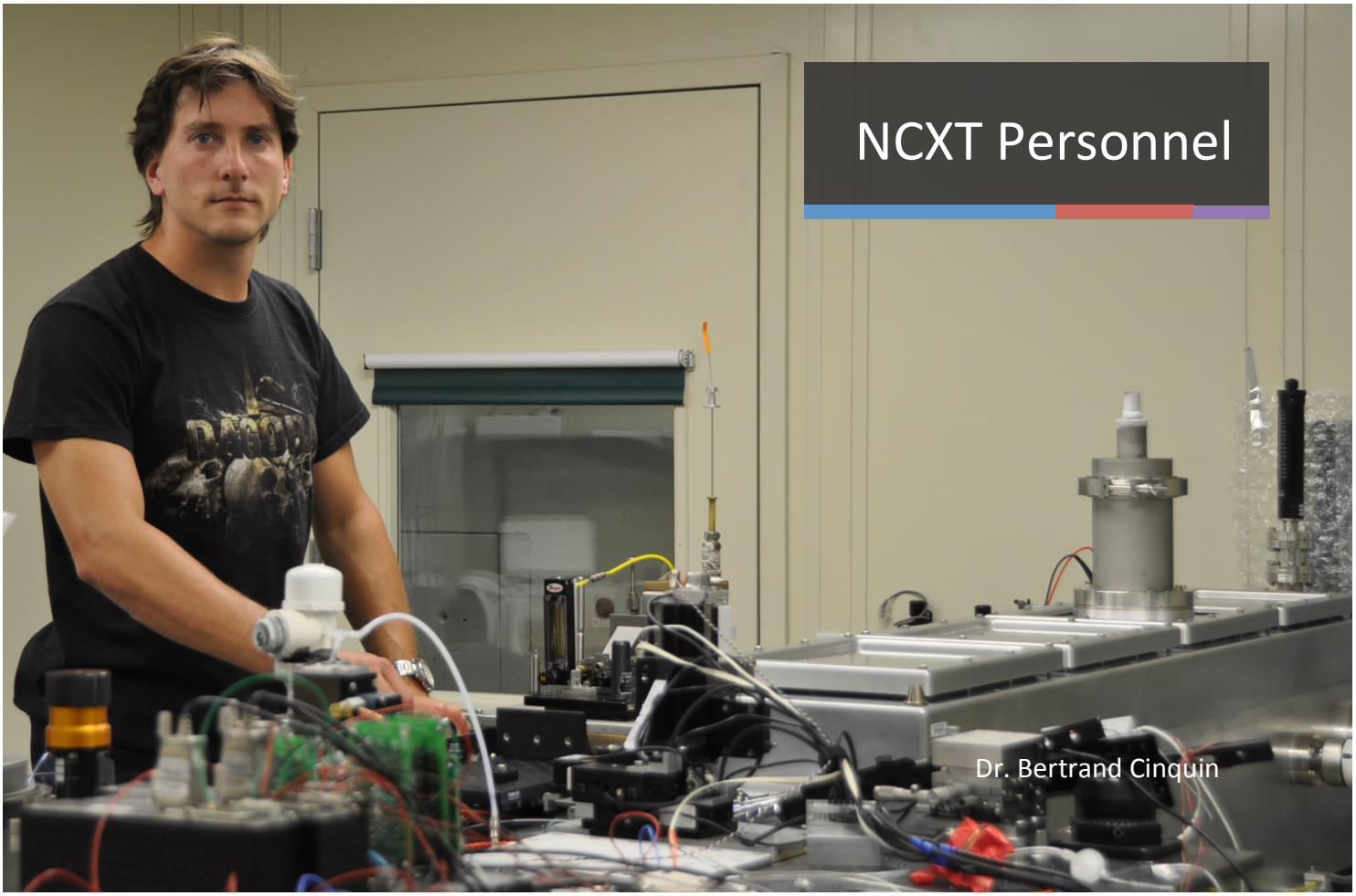

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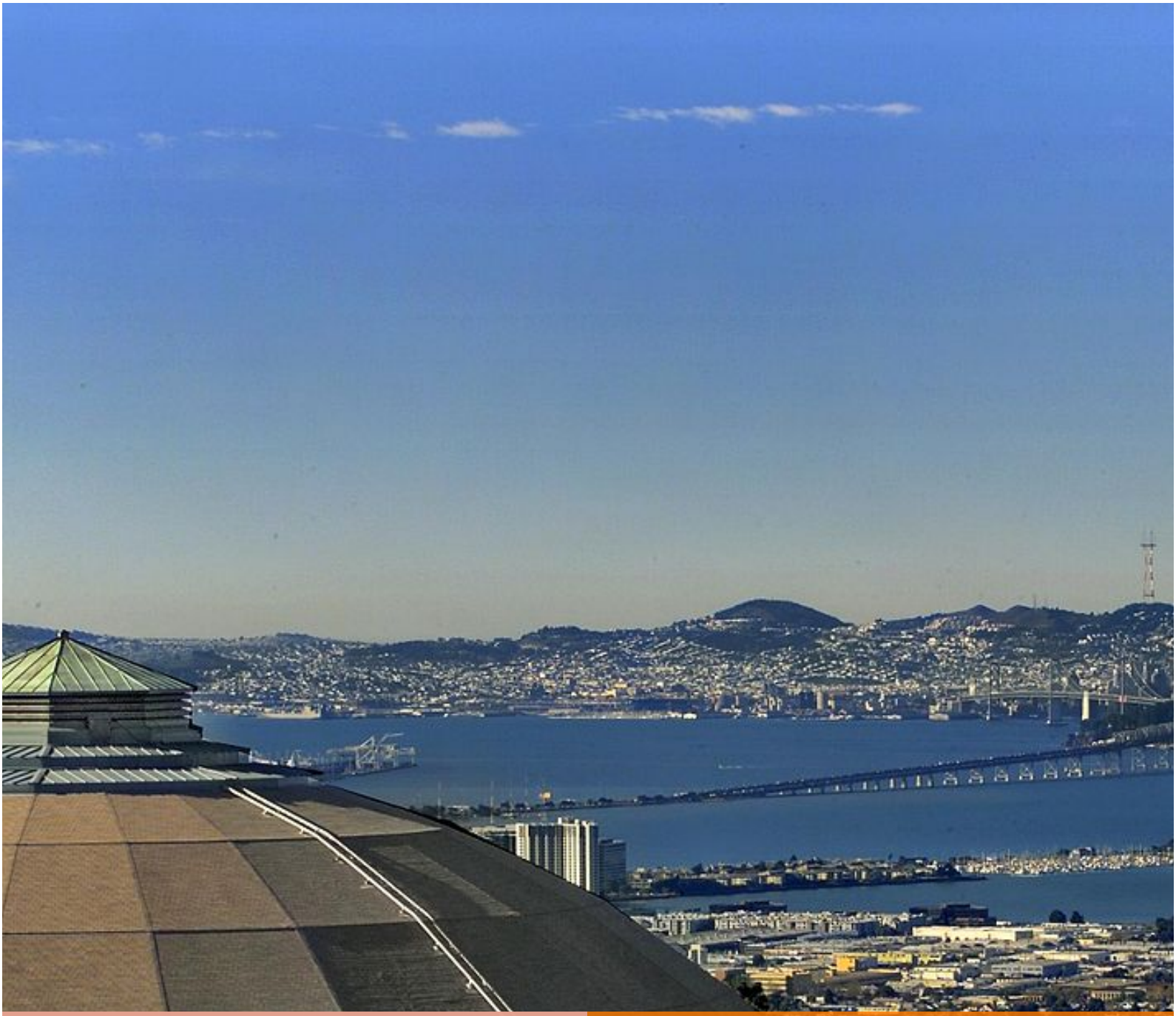

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